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NATURE AND CHANGES OF ORGANIC MATTER IN DEEP OCEAN CORES

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INTRODUCTION

Funding for this project began in 1969. In the first year, many of the techniques were established and equipment was calibrated and tested. Studies were undertaken in collaboration with J.W. Smith on the removal and identification of carbon compounds in several carbonaceous meteorites. The results do show that in type I and type II chondrites, the bulk of the carbon must be indigenous, whereas in type III and non-carbonaceous chondrites, volatile extractable organic molecules are probably derived from contamination. The criteria were established by comparing C^{13}/C^{12} ratios in compounds removed from the meteorites and in compounds extracted from terrestrial sediments.

These criteria were tested shortly after publication of the data, through analysis of the Murchison meteorite which fell in Victoria, Australia in September, 1969. The study carried out in collaboration with Ames Research Center, gave strong support for the extraterrestrial origin of the hydrocarbons and amino acids. The C^{13}/C^{12} data gave strong support to other data on the distribution pattern of these compounds, and indicated that they could not have originated from recent terrestrial contamination of known composition. We believe this finding may be one of the most important to have been published in the field of biological studies on meteorites. It now opens new avenues of endeavor into investigation of extraterrestrial samples.

A study was also undertaken in collaboration with Mrs. Mary Jo Baedeker to investigate the origin of some isoprenoid hydrocarbons in sediments from the hypersaline lake, the Dead

Sea. These hydrocarbons, the best-known being phytane (2, 6, 10, 14-tetramethylhexadecane) and pristane (2, 6, 10, 14-tetramethylpentadecane) are very common in ancient sediments. They are often considered to be derived from cleavage of the phytol side chain of the chlorophyll molecule, and have therefore frequently been used as evidence for biogenicity in ancient rocks. Strangely enough, however, the hydrocarbon phytane is very rare in recent sediments, and pristane has been found only in trace quantities. Chlorophyll begins to degrade rapidly in these sediments. In the Dead Sea, however, chlorophyll degrades much more slowly but phytane as well as pristane are present in the surface sediment in a significant ratio to the total hydrocarbon content of the sediment. One possible source for the hydrocarbons could be the red halophilic bacteria which dominate the biota of the water column. Since it is known that these organisms contain an ether-linked glycerol-diphosphatidyl lipid, it was considered that cleavage of the linkages would release phytol which would eventually be reduced into the isoprenoid hydrocarbon. A test for this lipid in the sediment showed it was present. The isoprenoid hydrocarbons most probably arose from this source rather than a chlorophyll side chain. This fact must therefore be kept in mind when considering the origin of molecules in ancient rocks in an attempt to find evidence for initiation of a photosynthetic mechanism. A detailed report of the study is enclosed.

During 1970-1971, the sediment from five deep sea cores were examined, representing fifteen individual samples. Some of the sediment was typical of normal deep sea clays, low in organic matter

(<0.2% organic C), whereas others were richer and approached more closely near shore environments, containing about 1.0% organic carbon. Two cores originated in the Gulf of Mexico, and the influence of upward migration of petroleum can be noticed from the unusually high hydrocarbon values. The cores of others came from the west Atlantic Ocean. The samples represent an age spread Pleistocene (<100,000 years) to Eocene (>50 X 10⁶ years). A more detailed summary of the results is given below:

Papers Published Under Support of Grant

1. J.W. Smith and I.R. Kaplan. Endogenous Carbon in Carbonaceous Meteorites. Science, 167, 1367-1370 (1970).
2. I.R. Kaplan and M.J. Baedeker. Evidence for Phosphatidyl Glycerophosphate Lipid in Hypersaline Sediment from Dead Sea. Israel J. of Chemistry, 8, 529-533, (1970).
3. K. Kvenvolden, J. Lawless, K. Pering, E. Peterson, J. Flores, C. Ponnamperna, I. Kaplan and C. Moore. Evidence for Extraterrestrial amino-acids and Hydrocarbons in the Murchison Meteorite. Nature, 228, 923-926, (1970).

Organic Constituents of Deep Sea Sediments

Studies were performed on fifteen sediment samples to separate, identify and quantitatively determine the following organic components:

- (i) Hydrocarbons - straight-chain paraffinic, branched chain isoprenoid, aromatic (perylene).
- (ii) Fatty Acids - free and soluble in organic solvent
- bound and soluble after hydrolysis.
- (iii) Alcohols - mostly bound as esters
- (iv) Chlorins and Porphyrins

All of these compounds have been examined by various authors interested in determining evidence for the presence of life in ancient Proterozoic rocks. The data found are presented in Tables 1-11 and the techniques used for removal, detection and estimation are given in the last section.

It is evident from Table 1 that the samples studied represent a wide range of ages, depth of burial and organic carbon content. The separate hydrocarbons, fatty acids and alcohols isolated are shown in Tables 2 to 9 for the individual samples analyzed. Tables 10 and 11 summarize the results in terms of the absolute quantities isolated and in terms of their content relative to the organic content of the sediment.

Several interesting and somewhat surprising results are apparent and are summarized below:

1. The average value for the extractable components in the sediment is 0.063% of organic carbon, compared with 0.23 for

near shore organic rich sediments and 0.92 for Dead Sea sediment. This suggests that the lipid-soluble components are actually being removed and not contributed as one may expect during aging.

2. Following the above, there is no strong evidence for hydrocarbon (hence petroleum) formation during low temperature and slow diagenesis.
3. Aromatic hydrocarbons form only under special circumstances. For example, perylene was only detected in core #26.
4. Free fatty acids do not appear to persist, except under special circumstances.
5. Pristane and phytane are generally only present in small quantities. The ratio Pr/Ph is generally >1 although it can be <1 (as in core #27). Thus, these isoprenoid hydrocarbons are formed in significant quantities only under special conditions.
6. Chlorins do not necessarily disappear rapidly. The oldest sediment we have detected chlorins in, is about 20×10^6 years old. However, porphyrins are not always present. In general, however, chlorins could not be detected in the oldest sediment, and apart from core #6 (taken from a deep sea oxidizing environment) porphyrins were detected in the absence of chlorins.
7. C^{13}/C^{12} ratios of the total organic carbon shows a range of distribution from $\delta C^{13} = -22\%$ (representing marine plankton as a source) to -27% (typical of land plants). It thus appears from these preliminary data that the source of origin of the organic matter is both marine and terrigenous.

8. The CPI index (carbon preference ratio) of the straight chain alkanes is always >1 , indicating that the hydrocarbons are immature and have formed biochemically and possibly by one carbon decarboxylation of fatty acids.

The results summarized above clearly show that preservation of organic matter has taken place in core #26 to a greater extent than any of the others. This fact is important, and an understanding of the environment of formation may eventually enable us to predict what factors are important for preservation of organic molecules. A consequence of this knowledge will be to determine which ancient samples of Proterozoic rocks can be studied with reliability.

TABLE 1. Descriptive data, organic carbon content and δC^{13} of analyzed samples.

Sample Hole	Depth(m)	JOIDES designation ¹	Age	Description	% Org. C	δC^{13} (‰)
2	20	1-2 (0-65)	Pleist.	Calcareous mud	0.45	-23.4
2	103	4-1 (30-90)	Pliocene	Cocc. ooze, H ₂ S	0.38	-22.8
6A	15	1-2 (10-72)	Pleist.	Brown clay	0.13	-24.0
6	43	1-1 (90-150)	Pliocene	Brown clay	0.16	-25.4
6	153	2-2 (0-70)	Eocene	Gray-green clay	0.09	-25.7
3	34	1-2 (0-72)	Pleist.	Calc. Silt, clay	1.11	-26.6
3	209	4-1 (33-100)	Pleist.	Cocc. ooze, clay	0.82	-21.7
3	324	5-2 (102-150)	Pliocene	Cocc. ooze, clay	0.47	-22.1
3	534	9-2 (18-60)	Miocene	Grayish-green silty clay	0.47	-22.2
26	100	1-3 (81-130)	Pleist.	Gray silty clay	0.87	-25.3
26	230	3-2 (100-150)	Pleist.	Olive-gray clay-claystone	1.00	-27.0
26	478	5-1 (89-131)	Pleist.	Dk. olive gray silty claystone	0.51	-25.2
27	143	2-2 (95-150)	Miocene	Lt. olive gray clay	0.27	-25.0
27	237	3-2 (0-64)	Miocene	Stiff green-gray clay	0.58	-26.8
27	249	4-3 (84-150)	Miocene	Green-yellow mottled clay	0.18	-24.5

¹Core-section (interval)

TABLE 2

DISTRIBUTION OF NORMAL HYDROCARBONS AND FATTY ACIDS
IN JOIDES HOLE 2 ($\mu\text{g}/100\text{g}$ dry sediment)

Hydrocarbons			Hydrolyzable Fatty Acids	
C#	20m	103m	20m	103m
14	1.2	2.9	0.9	tr
15	1.9	3.5		
Δ -16			tr	
16	2.4	4.4	11.9	1.4
17	2.8	5.0		
Δ -18			2.9	0.3
18	3.5	4.5	4.2	0.4
19	3.9	3.3	0.2	
20	3.5	2.7	1.9	0.6
21	3.6	1.3	1.1	
22	3.6	1.3	3.9	1.1
23	6.3	1.7	1.2	
24	5.8	1.4	3.7	0.8
25	11.3	2.4	0.9	
26	26.2	4.0	6.1	1.6
27	86.4	7.9	1.3	
28	115.5	14.3	7.6	1.2
29	511.7	95.0	tr	
30	117.3	21.9	12.0	
31	525.9	142.7		
32	49.4	19.7		
33	217.3	63.2		
Total	1699.5	403.1	59.8	7.4
CPI_{21-31}	3.88	4.64		
<u>Pristane</u>	2.33	1.35		
<u>Phytane</u>				

Free Fatty Acids - none identified

Alcohols - $\sim 7 \mu\text{g}/100\text{g}$ in 20 and 103 m, traces 14, 16, 18, 20, 22, 24, 26.

TABLE 3
DISTRIBUTION OF HYDROCARBONS
IN JOIDES HOLE 3 ($\mu\text{g}/100\text{g}$)

C#	34M	209m	324m	534m
15	42.5	tr	tr	0.5
16	50.1	tr	0.8	0.6
17	57.2	1.1	1.8	1.0
18	47.8	1.2	1.6	1.0
19	56.3	1.6	2.1	1.1
20	61.6	1.0	1.6	1.0
21	55.8	1.3	2.1	1.7
22	34.4	1.3	3.7	1.9
23	55.8	1.6	3.0	2.7
24	38.4	1.8	2.9	1.8
25	79.7	4.5	7.3	4.7
26	42.7	5.4	5.6	2.7
27	92.0	7.0	13.3	10.6
28	23.6	2.4	6.3	4.5
29	88.0	12.4	22.6	33.0
30	16.6	3.3	6.5	6.8
31	87.5	13.5	32.9	26.7
Total	930.0	59.4	114.1	102.3
Pristane	66.5	1.1	1.9	1.1
Phytane	38.3	1.4	1.1	1.1
CPI ₂₁₋₂₉	2.08	2.05	2.14	3.56
Pr/Ph (1)	1.73	.79	1.73	1.00
Pr/Ph (2)	2.00	.76	1.14	.91

(1) ApL analytical column

(2) ApL capillary column

TABLE 4

DISTRIBUTION OF FATTY ACIDS AND ALCOHOLS

IN JOIDES HOLE 3 ($\mu\text{g}/100\text{g}$)

C#	HFA				ALCOHOL			
	34m	209m	324m	534m	34m	209m	324m	534m
14	8.1	3.0	7.1	3.7	3.3	1.0	1.4	3.2
Δ 16	5.2	1.3	1.2	2.2				
16	42.9	15.4	11.8	16.4	2.0	0.9	1.0	1.5
Δ 18	7.4	2.8	9.5	7.3				
18	12.3	3.6	3.6	5.4	1.9	0.9	0.7	1.6
20	7.4	1.1	0.6	2.5	15.5	2.3	+	2.5
22	4.0	1.0	0.6	2.0				
24	3.1	1.0	1.5	1.7				
Total	90.4	29.2	35.9	41.2	22.7	5.1	3.1	8.8
Δ 16/16	.12	.08	.10	.13				
Δ 18/18	.60	.78	2.64	1.35				

Free fatty acids - $< 1 \mu\text{g}$

Hydrolyzable fatty acids - samples at 34 and 324 m had very large peaks $> C_{20}$ (not definitely identified); small amounts of odd f. a. present in all samples.

Alcohols - phytol identified in only 34 m sample ($0.9 \mu\text{g}$); no dihydrophytol; large peaks $> C_{20}$ present.

TABLE 5

DISTRIBUTION OF HYDROCARBONS
IN JOIDES HOLE 6 ($\mu\text{g}/100\text{g}$)

C#	15m	43m	153m
17		0.24	
18	0.30	0.42	0.16
19	0.15	0.54	0.30
20	0.19	0.52	0.31
21	0.19	0.34	0.29
22	0.54	0.36	0.32
23	0.35	0.20	0.32
24	1.56	0.21	0.34
25	0.65	0.33	0.47
26	0.53	0.29	0.49
27	1.25	0.67	0.65
28	0.52	1.16	0.64
29	2.10	4.40	2.28
30	0.48	1.61	0.62
31	2.54	9.36	2.77
32	0.45	0.96	0.37
33	1.49	2.32	1.66
Total	13.29	23.93	11.99
CPI_{25-31}	2.58	4.05	2.93

Pristane and Phytane not identified.

Free fatty acids - 2 unidentified peaks < 1 μg .

Hydrolyzable fatty acids - stray peaks < 5 μg .

TABLE 6

DISTRIBUTION OF HYDROCARBONS IN JOIDES HOLE 26

 $(\mu\text{g}/100\text{g})$

C #	100m	230m	478m
16	----	4.9	0.4
17	1.3	1.5	0.3
18	1.1	1.4	24.2
19	2.6	2.0	5.0
20	1.7	2.0	4.0
21	3.3	5.3	6.1
22	4.4	4.5	6.8
23	+	12.0	10.2
24	9.2	6.4	9.7
25	8.9	15.0	18.4
26	5.8	6.2	9.5
27	14.6	18.7	21.7
28	7.8	8.3	10.9
29	37.5	46.3	8.3
30	8.8	10.7	17.7
31	48.1	51.1	67.9
32	13.2	13.4	22.3
33	22.9	32.4	78.7
Total	191.2	242.1	322.1
CPI ₂₅₋₃₁	3.25	3.74	2.15

TABLE 7

DISTRIBUTION OF FATTY ACIDS AND ALCOHOLS IN JOIDES HOLE 26 ($\mu\text{g}/100\text{g}$)

	FFA			HFA			Alcohols		
C#	100m	230m	478m	100m	230m	478m	100m	230m	478m
12	1.2	3.7	0.2	--	--	--	--	--	--
13	0.5	0.7	--	--	--	--	--	--	--
14	0.2	1.4	0.3	2.7	1.7	0.4	--	--	--
15	--	0.6	0.2	--	--	--	--	--	--
$\Delta 16$	--	--	--	3.4	1.4	--	--	--	--
16	0.5	3.8	1.6	23.8	19.4	9.1	0.8	2.3	0.5
17	--	0.3	0.1	--	--	--	--	--	--
$\Delta 18$	--	--	--	4.6	3.5	1.4	--	--	--
18	0.2	1.4	1.3	8.7	3.8	5.2	1.1	2.5	0.8
19	--	--	0.3	--	--	--	--	0.5	--
20	0.1	0.3	--	4.9	5.7	1.8	2.3	7.5	1.2
21	--	--	--	--	--	--	1.7	3.8	--
22	2.3	3.5	0.8	12.1	8.4	4.4	30.5	76.5	8.6
23	1.2	1.8	0.3	5.2	--	--	5.9	11.3	1.5
24	1.7	21.6	2.4	48.4	29.6	12.2	64.6	116.6	15.7
25	0.8	6.2	0.7	11.5	--	--	7.0	12.5	1.6
26	1.5	25.4	2.4	36.8	24.9	7.4	47.3	77.8	11.1
27	--	4.5	0.5	69.1	--	--	3.5	5.9	0.9
28	2.0	13.7	1.6	17.2	15.5	3.7	28.1	43.0	8.0
29	0.5	3.2	0.2	--	--	--	--	--	--
30	2.7	7.5	1.2	92.1	10.8	3.2	39.8	46.9	10.5
Total	15.4	99.6	14.1	340.5	129.7	48.8	232.6	407.1	60.4

Fatty acids - odd C# acids > C₂₀ present in ~1-2 μg amounts not reported above.

Alcohols - found with FFA in following amounts: 100m-21.7; 230m-14.1; 478m-2.9 $\mu\text{g}/100\text{g}$.

TABLE 8

Distribution Hydrocarbons
in JOIDES HOLE 27

C#	143m	237m	249m
17	0.04	0.22	0.17
18	0.14	0.36	0.30
19	0.12	0.43	0.43
20	0.23	0.57	0.50
21	0.14	0.78	0.52
22	0.24	0.86	0.76
23	0.30	2.31	0.90
24	0.40	1.51	0.66
25	0.47	4.12	1.69
26	0.59	2.02	0.93
27	0.79	5.05	2.32
28	0.56	2.46	1.29
29	1.72	11.01	4.48
30	0.54	3.47	1.45
31	2.80	22.77	6.90
32	0.89	6.35	2.79
33	1.77	15.88	5.23
Pr	0.04	0.22	0.13
Ph	0.21	0.51	0.30
Total	11.99	80.90	31.75
CPl ₂₁₋₃₁	2.15	3.34	2.50
Pr/Ph	0.19	0.43	0.43

TABLE 9

DISTRIBUTION OF HFA and ALCOHOLS

IN JOIDES HOLE 27

 $\mu\text{g}/100\text{g}$

C# Depth (m)	HFA			ALCOHOLS		
	143	237	249	143	237	249
14	0.2	7.5	1.4			
15	-	1.9	0.7			
Δ -16	0.2	5.2	1.9			
16	1.2	65.5	15.3	+	1.0	+
17	-	0.7	+			
Δ -18	0.4	15.3	1.6			
18	0.5	19.9	3.0	+	1.8	+
19	-	0.2	-	+		
20	0.2	0.9	+	+	1.6	+
21	-	0.3	-	+		
22	+	3.0	0.5	+	34.6	
23	-	1.2	+	+	+	
24	+	27.2	0.8	+	42.9	
25	-	5.3	-		+	
26	+	49.6	1.5	+	39.6	
27	-	4.4	+		+	
28	-	30.9	1.3		14.9	
29	-	2.3	-			
30	-	8.8	-			
Total	2.7	250.1	28.0	~ 9.2	136.4	~ 3.8
Δ -16/16	0.17	0.08	0.12			
Δ -18/18	0.78	0.77	0.52			

TABLE 10. SUMMARY OF DATA
($\mu\text{g}/100\text{g}$)

Hole	Depth (m)	H.C.	Perylene	FFA	HFA	Alc.	Chlorins	Porphyrins
2	20	1699	a.	a.	60	~ 7	a.	1.9
2	103	403	a.	a.	7	~ 7	a.	0.7
6A	15	13	a.	a.	< 5	-	a.	a.
6	43	24	a.	a.	< 5	-	a.	a.
6	153	12	a.	a.	< 5	-	a.	a.
3	34	930	t.	a.	90	23	8.1	7.1
3	209	59	a.	a.	29	5	4.9	0.9
3	324	114	a.	a.	36	3	0.8	1.0
3	534	102	a.	a.	41	9	a.	2.3
26	100	191	18.5	15	341	233	56.8	a.
26	230	242	33.1	100	130	407	14.6	a.
26	478	322	16.1	14	49	60	a.	1.8
27	143	12	a.	a.	3	9	a.	0.2
27	237	81	a.	a.	250	136	1.2?	a.
27	249	32	a.	a.	28	4	a.	0.8

a. = none identified
t. = trace

TABLE 11. ORGANIC CONSTITUENTS IDENTIFIED IN JOIDES SAMPLES

% of total Org. C. $\times 10^2$

Hole	Depth (m)	H.C.	Perylene	FFA	HFA	Alc.	Chlorins	Porphyrins
2	20	32.16	a.	a.	1.04	~ 0.13	a.	0.03
2	103	9.03	a.	a.	0.14	~ 0.15	a.	0.01
6A	15	0.85	a.	a.	< 0.30	- ?	a.	a.
6	43	1.28	a.	a.	< 0.30	- ?	a.	a.
6	153	1.14	a.	a.	< 0.30	- ?	a.	a.
3	34	7.14	a.	a.	0.63	0.17	0.05	0.05
3	209	0.61	a.	a.	0.28	0.05	0.04	0.01
3	324	2.06	a.	a.	0.60	0.05	0.01	0.02
3	534	1.85	a.	a.	0.68	0.16	a.	0.04
26	100	1.87	0.20	0.13	3.05	2.19	0.49	a.
26	230	2.06	0.32	0.78	1.01	3.32	0.11	a.
26	478	5.38	0.30	0.21	0.75	0.96	a.	0.03
27	143	0.38	a.	a.	0.09	0.27	a.	0.01
27	237	1.19	a.	a.	3.35	1.91	0.02	a.
27	249	1.51	a.	a.	1.21	0.18	a.	0.03

Identification of Organic Compounds
in Joides Sediment

Reagents and Instrumentation

Organic solvents used in the procedure were distilled and the level of solvent contamination determined as $<4 \times 10^{-9}$ g/ml for ether and $<4 \times 10^{-11}$ g/ml for all other solvents. The chromatographic packing materials, silicic acid (minus 325 mesh) and florisil (minus 100 mesh) were prewashed with the eluting solvents in reverse order of actual use. Evaporation of large quantities of solvents was achieved by rotary evaporation under vacuum. Small quantities of solvents were evaporated in a stream of nitrogen purified by passage through a copper tube heated to 550°C.

Gas chromatographic analyses were carried out on Varian Aerograph Models 204 and 1520 and Hewlett-Packard Model 5750 gas chromatographs. The following columns were used: 5' X 1/8", 3% SE-30 on 100-120 mesh Chromosorb W; 5' x 1/8", 2% Apiezon L on Chromosorb W; 5' x 1/8", 3% DEGS on Chromosorb W; 100' (.01" I.D.) capillary column coated with Apiezon L. Ultraviolet and visible spectra were obtained on a Cary 15 spectrophotometer, equipped with a 0.1cc microcell. A 21-491 CEC mass spectrometer coupled with a 1200 Varian gas chromatograph with a 5' x 1/8", 5% SE-30 column was used for structure determinations.

Samples

Sediment samples received from the JOIDES project had been squeezed to remove interstitial water and frozen in Aluminum

foil packages. The samples were stored frozen until analyzed. Samples from the following locations were analyzed: Leg 1, Hole 2,3,6 and Leg 4, Hole 26,27.

Organic carbon and C^{13}

The total organic carbon was determined by reacting a known weight of sediment with 0.2N HCl to remove carbonate. The residue was washed repeatedly with distilled water to remove all the acid and dried in a vacuum dessicator at 40°C. The dried sample was combusted in an oxygen atmosphere at 1100°C, and the volume of CO_2 measured to calculate the amount of organic carbon in the sediment. The CO_2 was collected and the C^{13}/C^{12} ratio measured on a Nuclide Co. 60°, mass spectrometer.

Extraction

A procedure was developed to determine in a single sample hydrocarbons, fatty acids, alcohols, chlorins, and porphyrins.

150 g wet sediment was extracted with 200 ml benzene: methanol (70:30) four times using a Virtis Model 45 homogenizer. If necessary, more methanol was added during the extraction to obtain adequate mixing of the solvent system and the sediment. The sediment was filtered by suction after each extraction and the combined filtrates reduced in volume by rotary evaporation until most of the organic solvents were removed. The remaining mixture was acidified with a few drops of HCl and extracted with benzene. The benzene extract was taken to near dryness by rotary evaporation and applied to a silicic acid column (1.9 x 15cm). The column was eluted with 75 ml each (1) hexane to remove hydrocarbons (2) hexane:ether (2:1) to remove fatty acids, esters, alcohols

and some pigments and (3) MeOH to remove any remaining pigments. The above fractions were divided, one part used for the study of pigments and that remaining for the analysis of hydrocarbons, fatty acids and alcohols.

Hydrocarbons

The hexane fraction from the silicic acid column was evaporated to near dryness and rechromatographed to separate aliphatic and aromatic hydrocarbons. The total hydrocarbon fraction was applied to a small florisil column (0.5 x 4cm) and eluted with 5 ml hexane and then with 5 ml benzene. The hexane eluent containing aliphatic hydrocarbons and the benzene eluent containing aromatic hydrocarbons were analyzed by gas chromatography. In addition the aromatic fraction was analyzed by gas chromatography-mass spectrometry and examined by UV-Visible spectrophotometry.

Fatty acids and Alcohols

The hexane-ether fraction from the silicic acid column contained the free fatty acids and esters. The solution was evaporated to near dryness and the residue taken up in benzene. The free fatty acids were separated from the esters by extraction with 0.1N NaOH. The basic solution was then acidified and the free fatty acids re-extracted into benzene.

The esters were refluxed in 10% KOH in methanol for 3 hours. Water was added to the hydrolyzate and the alcohols extracted with benzene:hexane (1:1), the hydrolyzed fatty acids remaining in the aqueous phase. The aqueous hydrolyzate was acidified with HCl and the fatty acids extracted into benzene:hexane.

The free fatty acid and hydrolyzable fatty acid fractions were taken to near dryness, re-suspended in 3 ml 5% MeOH in ether, and methylated with diazomethane (Schlenk and Gellerman,

1960). The free fatty acid methyl ester, hydrolyzable fatty acid methyl ester, and alcohol fractions were analyzed by gas chromatography.

The presence of unsaturated fatty acids was verified by hydrogenation of the sample using PtO_2 as a catalyst. The subsequent analysis by gas chromatography showed the elimination of the unsaturated acid and a corresponding increase in the amount of saturated compound.

Schlenk H. and Gellerman J.L. (1960). Esterification of fatty acids with diazomethane on a small scale. Anal. Chem. 32, 1412-1414.

PERYLENE

Qualitative and quantitative determination

Fraction I (hexane on silicic acid) contains all of the perylene and other compounds. A second chromatography using hexane:benzene mixtures on silicic acid or silica gel columns will separate most of the other compounds that have an absorption between 400-500 m μ . (e.g. carotenoids and some low molecular aromatics), from the perylene.

The perylene fraction can be detected by its very intensive fluorescence and typical UV spectra (Orr and Grady, 1967).

In order to determine the amount of perylene and identification, two major techniques were applied:

(1) UV - The importance of the second column chromatography is shown by this method. If one compares the total UV spectra of Fraction I to that of the rechromatographed perylene it is obvious that for quantitative determination the latter is much more accurate.

Out of the four typical peaks in the perylene UV spectrum the highest $\lambda_{\text{max}}^{\text{benzene}}$ 440 m μ was selected. $\epsilon^* = 4.06 \times 10^4$ (Schnurmann et. al., 1953).

(2) GLC and GLC-MS - The fraction containing perylene is chromatographed on a 5' x 1/8" OV-17 column, temperature programmed 200-300° C. at a rate of 12°/min.

In most cases if perylene is present, it is the major peak in that fraction's chromatogram. For further identification a standard perylene sample is coinjected.

Some of the more enriched samples were checked by the combination of a GC and medium resolution mass spectrometer (CEC 21-491). The mass spectrum shows that the perylene peak

on the GLC is at least 95% non-substituted perylene. That was proven by comparing the spectrum with standard perylene spectrum.

References

Orr W.L. and Grady J.R. (1967) Perylene in basin sediments off southern California. Geochim. Cosmochim. Acta 31, 1201-1209.

Schnurmann R., Maddams W.F. and Barlow M.C. (1953) Spectrophotometric identification of polynuclear aromatic components in high boiling petroleum fraction. Anal. Chem. 25, 1010-1013.

* units for extinction coefficient are 1/mole/cm

Pigments determination

After chromatographing on silicic acid one-half of the three fractions is checked by UV (Cary 15).

Fraction I (Hexane)- Contains mostly the aliphatic hydrocarbons but in some recent sediments one might find carotenoids and some aromatic compounds. In order to separate the aliphatic hydrocarbons from the carotenoids and aromatics, we re-chromatographed this fraction on Florisil (-100 mesh). Elution with pure hexane (without hexene) will give the first fraction containing the aliphatic hydrocarbons. The second fraction is eluted with benzene and contains the carotenoids and aromatics.

The use of intermediate mixtures of benzene and hexane can result in a separation between the carotenoids and some of the aromatic compounds, such as perylene.

The determination of perylene is discussed separately. Carotenoids were determined quantitatively by UV measurements, using $\epsilon = 13.8 \times 10^4$ (Zechmeister and Polgar, 1943) for the major peak at 452 m μ . The only sample that contained carotenoids (in this study) was the Mexico Bay mud, all of the JOIDES samples lacked that group of pigments.

Fraction II (Hexane-ether)- This fraction contains most of the organic functional substituted compounds e.g. fatty acids, esters ...

The pigments in this fraction are primarily porphyrins. Sometimes when the sample tested is very rich in chlorins, there is a chance that some of them, will appear in this fraction too. Thus in some cases it will be necessary to re-chromatograph fraction II.

Fraction III (MeOH)- This fraction is highly impure and contains the rest of what was left on the column (silicic acid).

The asphaltic matter contains most of the chlorins. The chlorins can be washed out after evaporating the methanol by hexane. However, that will not extract all of the chlorins.

Fractions II and III are checked by UV and if there is the slightest doubt of a mixture, we recombined both fractions and re-chromatographed them on silica gel.

Re-chromatography of Fractions II and III on Silica Gel

The column is prepared with hexane as the solvent.

Eluents:

- (1) Hexane - no pigments
- (2) Hexane:benzene (50:50) some aromatic compounds
- (3) Hexane: benzene (20:80) porphyrins
- (4) Benzene - porphyrins

Fraction 3 will be less substituted as to the carbonilic groups, while fraction 4 will show a higher carbonyl absorption (IR studies). In some cases fraction 3 and 4 might differ also in the type of metal complexed with the porphyrin.

- (5) Benzene and Chloroform - 5% to 20% -Chlorins. The more chloroform used the more carbonyl type absorbance is shown in the IR.

Quantitative calculation

The average MW=600 for porphyrins,
using the Soret Peak 390-408 $m\mu$, $\epsilon = 4 \times 10^5$ (Hodgson, et. al., 1968).

The average MW=603 for Chlorins. This was determined as to the mixture of the chlorins starting from chlorophyll to pheophorbide. UV data calculated for 660 $m\mu$, $\epsilon = 3 \times 10^4$ (Goedheer, 1966). All the UV spectra were measured in benzene.

References

Goedheer J.C. (1966) Visible absorption and fluorescence of chlorophyll and its aggregates in solution. In: The Chlorophylls Ed. L.P. Vernon and R.S. Gilbert, Academic Press, New York, pp. 147-184.

Hodgson G.W., Hitchon B., Taguchi K., Baker B.L. and Peak E. (1968) Geochemistry of porphyrins, chlorins and polycyclic aromatics in soils, sediments and sedimentary rocks. Geochim. Cosmochim. Acta 32, 737-772.

Zechmeister L. and Polgar A. (1943) Cis-trans isomerization and spectral characteristics of carotenoids and some related compounds. J. Am. Chem. Soc. 65, 1522-1528.